I hereby certify that this correspondence is being electronically filed in the United States Patent and Trademark Office on September 2, 2008.

Jeff Lloyd, Patent Attorney, Reg. No. 35,589

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322
Docket No. MPS 4-87FD3
Patent No. 7,214,861

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Henk J. Franssen, Anton H.J. Bisseling
Issued	:	May 8, 2007
Patent No.	:	7,214,861
For	:	ENOD2 Gene Regulatory Region

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:	**Application Reads:**
Title page, Item (75):	Joint Declaration for Patent Application:
"Blsseling"	--Bisseling--
Column 3, line 5:	Page 6, line 12:
"an Mr of"	--M_r of--

Column 4, lines 8-9:	Page 9, line 7:
"Apro- bacterium"	--Agrobacterium--
Column 5, line 1:	Page 11, line 14:
"Example"	--Examples--
Column 5, line 5:	Page 11, line 18:
"sends"	--genes--
Column 5, line 8:	Page 11, line 21:
"Bnod2b"	--Enod2b--
Column 6, line 9:	Page 3, line 7 of Amendment After Allowance under 37 CFR§1.312 dated February 14, 2007:
"polvoentides"	--polypeptides--
Column 6, line 14:	Page 3, line 10 of Amendment After Allowance under 37 CFR§1.312 dated February 14, 2007:
"clone, cap be"	--clone, can be--
Column 6, line 26:	Page 3, line 20 of Amendment After Allowance under 37 CFR§1.312 dated February 14, 2007:
"oflhe"	--of the--
Column 6, line 32:	Page 3, line 25 of Amendment After Allowance under 37 CFR§1.312 dated February 14, 2007:
"nucleodde"	--nucleotide--

Column 6, line 35:	Page 3, line 26 of Amendment After Allowance under 37 CFR§1.312 dated February 14, 2007:
"nicleotides"	--nucleotides--
Column 6, line 45:	Page 14, line 22:
"nodidin"	--nodulin--
Column 6, line 51:	Page 15, line 3:
"regulatoTy"	--regulatory--
Column 6, line 61:	Page 15, line 13:
"oonsensus"	--consensus--
Column 7, line 3:	Page 15, line 22:
"detenuine"	--determine--
Column 7, lines 3-4:	Page 15, line 23:
"pres-sion"	--expression--
Column 7, lines 12-13:	Page 16, line 6:
"B. japoni- oum"	--B. japonicum--
Column 7, line 18:	Page 16, line 18:
"about 1 k:b"	--about 1 kb--
Column 7, line 26:	Page 16, line 19:
"Bnod2b"	--Enod2b--
Column 7, line 28:	Page 16, line 21:
"necleoticle"	--nucleotide--

Column 7, line 34:	Pages 16-17, lines 27 and 1:
"Bnod 2 gene"	--Enod2 gene--
Column 7, line 37:	Page 17, line 3:
"IS6S"	"1565"
Column 9, line 35:	Page 22, line 10:
"Mrs"	--M_rs--
Column 9, line 40:	Page 22, line 15:
"N-7S"	--N-75--
Column 9, line 41:	Page 5, line 24 of Amendment After Allowance under 37 CFR§1.312 dated February 14, 2007:
"ORF2FIGS. 2A-D"	--ORF2 on Figures 2A-D--
Column 9, line 42:	Page 22, line 18:
"praline-rieb"	--proline-rich--
Column 9, line 44:	Page 22, line 20:
"SDS-polyacrylamid. gels"	--SDS-polyacrylamide gels--
Column 9, line 47:	Page 22, line 22:
"N-7S"	--N-75--
Column 9, line 48:	Page 22, line 24:
"promo content"	--proline content--
Column 9, line 49:	Page 22, line 24:
"paUern"	--pattern--

Column 9, line 64:	Page 23, line 14:
"conosponding"	--corresponding--
Column 9, line 66:	Page 6, line 9 of Amendment After Allowance under 37 CFR§1.312 dated February 14, 2007:
"determined FIG. 2A-D"	--determined (Figures 2A-D--
Column 9, line 67:	Page 23, line 17:
"turned Enod2a"	--termed Enod2a--
Column 10, line 1:	Page 23, line 18:
"AtG"	--ATG--
Column 10, line 8:	Page 23, line 25 – page 24, line 1:
"11543. +– 0.20"	--1543 $\pm$ 20--
Column 16, line 16:	Page 7, line 17 of Amendment After Allowance under 37 CFR§1.312 dated February 14, 2007:
"merhionine"	--methionine--
Column 16, line 18:	Page 7, line 18 of Amendment After Allowance under 37 CFR§1.312 dated February 17, 2007:
"Its translation"	--its translation--
Column 16, line 19:	Page 7, line 19 of Amendment After Allowance under 37 CFR§1.312 dated February 14, 2007:
"^{358}-methionine"	--^{35S}-methionine--.

A true and correct copy of pages 6, 9, 11, 14, 15, 16, 17, 22, 23, and 24 of the specification as filed, a true and correct copy of Amendment After Allowance under 37 CFR§1.312 dated February 14, 2007, and a true and correct copy of Joint Declaration which support Applicants' assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,

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JL/gyl/mrc/trt/jb/abt
Attachments: Copy of pages 6, 9, 11, 14, 15, 16, 17, 22, and 23 of the specification as filed;
Copy of Amendment After Allowance under 37 CFR§1.312 dated February 14, 2007;
Copy of Joint Declaration; and
Official Certificate of Correction

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,214,861

APPLICATION NO.: 10/751,014

DATED : May 8, 2007

INVENTORS : Henk J. Franssen, Anton H.J. Bisseling

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page,
Item (75), "Blsseling" should read --Bisseling--.

Column 3,
Line 5, "an Mr of" should read --M_r of--.

Column 4,
Lines 8-9, "Apro-bacterium" should read --Agrobacterium--.

Column 5,
Line 1, "Example" should read --Examples--.
Line 5, "sends" should read --genes--.
Line 8, "Bnod2b" should read --Enod2b--.

Column 6,
Line 9, "polvoentides" should read --polypeptides--.
Line 14, "clone, cap be" should read --clone, can be--.
Line 26, "oflhe" should read --of the--.
Line 32, "nucleodde" should read --nucleotide--.
Line 35, "nicleotides" should read --nucleotides--.
Line 45, "nodidin" should read --nodulin--.
Line 51, "regulatoTy" should read --regulatory--.
Line 61, "oonsensus" should read --consensus--.

MAILING ADDRESS OF SENDER:
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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,214,861

APPLICATION NO.: 10/751,014

DATED : May 8, 2007

INVENTORS : Henk J. Franssen, Anton H.J. Bisseling

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 7,
Line 3, "detenuine" should read --determine--.
Lines 3-4, "pres-sion" should read --expression--.
Lines 12-13, "B. japoni-oum" should read --B. japonicum--.
Line 18, "about 1 k:b" should read --about 1 kb--.
Line 26, "Bnod2b" should read --Enod2b--.
Line 28, "necleoticle" should read --nucleotide--.
Line 34, "Bnod 2 gene" should read --Enod2 gene--.
Line 37, "IS6S" should read --1565--.

Column 9,
Line 35, "Mrs" should read --M_rs--.
Line 40, "N-7S" should read --N-75--.
Line 41, "ORF2FIGS. 2A-D" should read --ORF2 on Figures 2A-D--.
Line 42, "praline-rieb" should read --proline-rich--.
Line 44, "SDS-polyacrylamid. gels" should read --SDS-polyacrylamide gels--.
Line 47, "N-7S" should read --N-75--.
Line 48, "promo content" should read --proline content--.
Line 49, "paUern" should read --pattern--.
Line 64, "conosponding" should read --corresponding--.
Line 66, "determined FIG. 2A-D" should read --determined (Figures 2A-D--.
Line 67, "turned Enod2a" should read --termed Enod2a--.

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,214,861

APPLICATION NO.: 10/751,014

DATED : May 8, 2007

INVENTORS : Henk J. Franssen, Anton H.J. Bisseling

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 10,
Line 1, "AtG" should read --ATG--.
Line 8, "11543. +– 0.20" should read --1543 +– 20--.

Column 16,
Line 16, "merhionine" should read --methionine--.
Line 18, "Its translation" should read --its translation--.
Line 19, "^{358}-methionine" should read --^{35S}-methionine--.

MAILING ADDRESS OF SENDER:
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A cDNA library prepared from mature (21 day) soybean root nodules infected with Bradyrhizobium japonicum has been analyzed for copies of mRNA transcripts of early (7 day) nodulin genes (Franssen et al. (1987) Proc. Natl. Acad. Sci. USA 84:4495-4499). These genes are expressed while the nodule structure is being formed. pEnod2, the cDNA clone whose insert encodes nodulin-75 (N-75) was sequenced. The 998 bp insert includes a short poly(A) tail, and encodes a proline-rich protein. Nodule mRNA of about 1200 nucleotides in length was hybrid-selected and translated in vitro to give two polypeptides each with an M_r of about 75 kDa. The coding capacity of the mRNAs is significantly less than 75 kDa, but proline-rich proteins, such as collagen, are known to have anomalous behavior on polyacrylamide gels (J.W. Freytag et al. (1979) Biochemistry 18:4761-4768). N-75 expression was first detected at day 7 of nodule development, when nodule meristem emerges through the root epidermis with apparent expression increasing up to about day 13. Expression was observed in R. fredii-induced ineffective nodules without infection threads or bacteroids, so N-75 is likely to be involved in nodule morphogenesis rather than in the infection process per se (H. Franssen et al. (1987) Proc. Natl. Acad. Sci. USA 84:4495-4599).

There is a growing understanding of the DNA sequence elements which control gene expression. The following

Odell et al. (1985), Nature 313:810-812, describe a stretch of about 100 bp 5' to the start site of the CaMV 35S transcript which is necessary for increasing the level of expression of a reporter gene in chimeric constructions. Two different transcription activating elements which can function in plants are derived from the 780 gene and the ocs gene of Agrobacterium tumefaciens T-DNA (W. Bruce and W. Gurley (1987) Mol. Cell. Biol. 7:59-67; J. Ellis et al. (1987) EMBO J. 6:11-16). Regulated enhancer-like elements include those believed to mediate tissue-specific expression and response to illumination (M. Timko et al. (1985) Nature 318:579-582; H. Kaulen et al. (1986) EMBO J. 5:1-8; J. Simpson et al. (1985) EMBO J. 4:2723-2729; J. Simpson et al. (1986) Nature 323:551-554; R. Fluhr et al. (1986) Science 232:1106-1112).

The molecular mechanisms which regulate the expression of nodulin genes are not yet defined. V.P. Mauro et al. (1985) Nucleic Acids Res. 13:239-249, have analyzed the 5' flanking sequences of three nodulin genes of soybean for conserved DNA sequence motifs. They found three conserved sequence motifs: consensus sequence a 5'-GTTTCCCT-3', consensus sequence b 5'-GGTAGTG-3', and consensus sequence c 5'-TCTGGGAAA-3'. Whether these sequences function in the regulation of the nodulin genes is not known, and if they do, the stimuli which elicit expression are not known. The molecular mechanisms

development than other nodulin genes. These regulatory regions are those of early nodulin genes (Enod2).

The Enod2 gene, encodes a nodulin-75, a polypeptide with an apparent molecular weight of about 75 kDa expressed during the early stages of nodule development. The Enod2a regulatory region extends about 1 kb 5' from the start of transcription of the gene. All the signals required for tissue-specific regulated gene expression are contained within this 1000 bp 5' flanking region. The Enod2a regulatory region controls the expression of a downstream structural gene in a tissue-specific manner in the cortex of developing soybean nodule early in the nodule development process.

Examples of tissue-specific early nodulin regulatory regions are found in the 5' flanking region of the soybean (Glycine max) Enod2a and Enod2b genes which encode N-75. The Enod2a regulatory region extends about 1 kb 5' from the transcription start of the genes. The regulatory region contains the nucleotide sequence from Table 1 extending from about nucleotide 520 to about nucleotide 1565. The Enod2b regulatory region extends about 1 kb 5' from the transcription start of the gene, from about nucleotide 1320 to about nucleotide 2365, as in Table 2. These regulatory regions direct the expression of a downstream gene in a tissue-specific manner in the developing root nodule.

tissue-specific expression of foreign structural genes not naturally occurring in soybean. Transformation of plant cells and tissue with exogenous or foreign DNA and regeneration of plants from transformed cells or tissue can be achieved by any means known to the art.

Brief Description of the Figure

Figure 1 gives a schematic restriction endonuclease map of the soybean Enod2a and Enod2b genes, and the regions which flank them. Schematic diagrams of CHA-6 (containing the Enod2a gene) and CHA-9 (containing the Enod2b gene) are given. The regions sequenced (Tables 1 and 2) of both clones are indicated. The region of approximately 100% homology between the two genomic clones is indicated, as are the regions of the clones homologous to the Enod2 cDNA clone. Restriction endonucleases are labelled as follows: H = HindIII, B = BamHI, S = Sau3A, E = EcoRI.

Detailed Description of the Invention

The following definitions are provided, in order to remove ambiguities to the intent or scope of their usage in the specification and claims.

The Enod2 gene described herein is an early nodulin gene of soybean (Glycine max), which encodes nodulin polypeptides with an apparent molecular weight of about 75 kDa, nodulin 75 (N-75). Two such genes are exemplified by

the Enod2a and Enod2b genes which are identified by the DNA sequences given in Tables 1 and 2, respectively.

The Enod2 regulatory region is the DNA sequence 5' and adjacent to the Enod2 coding sequence, which includes promoter sequences and promoter-associated sequences and controls tissue-specific expression of the Enod2 genes in soybean. The regulatory region extends about 1 kb upstream from the transcription start site of an Enod2 gene. All the signals required from tissue-specific regulated gene expression are contained in the approximately 1 kb 5' flanking region. Within this stretch of DNA are sequences with homology to the TATA and CAAT consensus sequences of eukaryotic promoters, and the nodulin gene consensus sequences a and c (V.P. Mauro et al. (1985), supra), which are believed to be involved in the regulation of the expression of nod genes expressed later than Enod2 during nodulation. There are also sequence motifs with homology to the SV40 enhancer core consensus sequence which are found in the regulatory region of the soybean Enod2a gene. There may also be other sequence elements which modulate the level of gene expression, which respond to stimuli from the B. japonicum, or which determine the tissue-specific expression in the developing soybean root nodule after inoculation with Bradyrhizobium japonicum. The expression of Enod2 genes controlled by the Enod2 regulatory region is tissue-specific in that it is limited to the cortex of

developing soybean root nodules. The Enod2 regulatory region controls early gene expression in the developing root nodule of soybean with expression beginning at about 7 days after seed planting and inoculation. Expression is induced by contact with soybean nodulating bacteria, such as B. japonicum. Enod2 gene expression also occurs in the ineffective nodules induced by strains of Rhizobium fredii. The Enod2a regulatory region is a DNA sequence which includes promoter sequences and promoter-associated sequences and controls the expression of the soybean Enod2a gene. The Enod2a regulatory region extends about 1 kb upstream from the Enod2a gene transcription start. This region is specifically identified by the DNA sequence in Table 1 from about nucleotide 520 to about nucleotide 1565. The Enod2b regulatory region is a DNA sequence which includes promoter sequences and promoter-associated sequences and controls the expression of the soybean Enod2b gene. The Enod2b regulatory region extends about 1 kb upstream from the Enod2b gene transcription start. This region is specifically identified by the DNA sequence in Table 2 from about nucleotide 1320 to about nucleotide 2365. These regulatory regions direct tissue-specific expression of a downstream structural gene, such that the gene is selectively expressed in the inner cortex of the developing root nodule in soybean. The Enod2 common regulatory region is the DNA sequence extending about 500 bases upstream of the transcription start site of an Enod2

gene. The Enod2 common regulatory region is exemplified by the homologous sequences of Enod2a and Enod2b extending from about nucleotide 1050 to about nucleotide 1565 (Table 1), and about nucleotide 1850 to about nucleotide 2365 (Table 2), respectively. This common regulatory region controls tissue-specific expression of downstream genes in the cortex of developing soybean root nodules.

Expression refers to the transcription and translation of a structural gene so that a polypeptide is made. Gene expression may be assessed by direct detection of the protein product, by protein electrophoresis or by immunological methods, for example. Alternatively, expression may be assessed by the detection of the mRNA products of transcription (i.e. by northern hybridizations). This method is particularly appropriate for the testing of transcriptional regulatory sequences because the effects of processes such as protein degradation are excluded.

Promoter refers to the DNA sequences at the 5' end of a structural gene which direct the initiation of transcription. Promoter sequences are necessary, but not always sufficient, to drive the expression of the downstream structural genes. The promoter itself may be a composite of segments derived from more than one source, naturally occurring or synthetic. Eukaryotic promoters are

sequences can be empirically determined in DNA hybridization experiments, such as those described in B. Hames and S. Higgins (1985) Nucleic Acid Hybridization, IRL Press, Oxford, UK.

pEnod2 was isolated from a cDNA library prepared with 21-day-old soybean root nodule RNA, using RNA from 10-day-old nodules as a probe. Thus, pEnod2 represents an early nodulin cDNA clone. The early nodulin encoded by pEnod2 was identified by hybrid-selecting nodule mRNA and translating in vitro. Two polypeptides, with apparent M_rs of 75000, were found and were each called N-75. The mRNAs homologous to pEnod2 were only about 1200 nucleotides long, with the capacity to encode a protein of at most about 45 kDa. Therefore the soybean-specific insert of pEnod2 was sequenced and the amino acid sequence of N-75 was deduced. Two ORFs of similar size were found (labelled ORF1 and ORF2 on Tables 1 and 2), one with about 20 methionines and the other a proline-rich sequence, with a repeating heptameric sequence. Because of the anomalous migration on SDS-polyacrylamide gels and because of the labelling patterns the two N-75s, it was concluded that the proline-rich coding sequence (ORF1) was that of N-75. It is believed that N-75 is involved in nodule morphogenesis because of its proline content and because of the pattern of expression in the developing nodule. N-75 appears at about day 7 after sowing and inoculation, and increases

through day 13; mRNA continues to be present at least through day 21. N-75 is also produced in the developing ineffective nodule of soybean inoculated with Rhizobium fredii USDA257. That leads to the conclusion that typical nodule structure with successful infection of the root by rhizobia is not absolutely required for Enod2 expression.

Hybridization studies have shown that there are Enod2 cDNA homologous sequences in Pisum sativum, Vicia sativa, Parasponia, and alfalfa. In pea, the nod genes or genes adjacent to the nod genes of Rhizobium leguminosarum are known to be involved in the expression of the Enod2-homologous gene (F. Govers et al. (1986) Nature 323:564-566).

Two soybean genomic clones corresponding to pEnod2 have been isolated and the DNA sequences of the coding and flanking regions have been determined (Tables 1 and 2). The genes, termed Enod2a and Enod2b, are essentially homologous from about 600 bp 5' to the ATG translation start codon through the coding region, which is not interrupted by introns, and through some 500 bp of 3' flanking sequence. Comparison of the genomic clones with the Enod2 cDNA sequence indicates that one or both of these genes are expressed in the developing root nodule. S1 mapping of the transcription start site led to the conclusion that the Enod2a start site is at nucleotide 1543

I hereby certify that this correspondence is being facsimile transmitted to the United States Patent and Trademark Office on February 14, 2007.

Jay M. Sanders, Patent Attorney
Patent Attorney

AMENDMENT AFTER ALLOWANCE UNDER 37 CFR §1.312
Patent Application
Examining Group 1638
Docket No. MPS 4-87FD3
Serial No. 10/751,014

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Russell P. Kallis, Ph.D.
Art Unit	:	1638
Applicants	:	Henk J. Franssen, Anton H.J. Bisseling
Serial No.	:	10/751,014
Conf. No.	:	1638
Filed	:	January 2, 2004
For	:	ENOD2 Gene Regulatory Region

Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

AMENDMENT AFTER ALLOWANCE UNDER 37 CFR §1.312

Sir:

Please amend the above-identified patent application as follows:

Amendments to the Specification:

Please amend the first paragraph on page 1, after the title, as follows:

This application is a continuation of ~~co-pending~~ application Serial No. 09/564,142, filed May 3, 2000 (now abandoned); which is a continuation application of application Serial No. 08/859,555, filed May 20, 1997 (now abandoned); which was a continuation application of application Serial No. 08/411,062; filed March 27, 1995 (now U.S. Patent No. 5,631,358); which was a continuation application of Serial No. 07/214,297; filed July 1, 1988 (now abandoned).

Please amend Paragraphs [0019]-[0020] on page 11, lines 14-25, and page 12, lines 1-10, as follows:

[0019] Examples of tissue-specific early nodulin regulatory regions are found in the 5' flanking region of the soybean (Glycine max) Enod2a and Enod2b genes which encode N-75. The Enod2a regulatory region extends about 1 kb 5' from the transcription start of the genes. The regulatory region contains the nucleotide sequence from ~~Table 1~~ Figure 2 extending from about nucleotide 520 to about nucleotide 1565. The Enod2b regulatory region extends about 1 kb 5' from the transcription start of the gene, from about nucleotide 1320 to about nucleotide 2365, as in ~~Table 2~~ Figure 3. These regulatory regions direct the expression of a downstream gene in a tissue-specific manner in the developing root nodule.

[0020] An additional example of a tissue-specific early nodulin gene regulatory region is the DNA sequence common to the 5' flanking regions of the soybean Enod2a and Enod2b genes. This regulatory element contains DNA sequence as given in ~~Table 1~~ Figure 2, extending from about nucleotide 1050 to about nucleotide 1565, or given in ~~Table 2~~ Figure 3, extending from about nucleotide 1850 to about nucleotide 2365. This regulatory region directs the expression of a downstream structural gene in a tissue-specific manner in the developing root nodule.

Please amend Paragraph [0024] at page 14, lines 6-16, as follows:

BRIEF DESCRIPTION OF THE FIGURES

[0024] FIG. 1 gives a schematic restriction endonuclease map of the soybean Enod2a and Enod2b genes, and the regions which flank them. Schematic diagrams of CHA-6 (containing the Enod2a gene) and CHA-9 (containing the Enod2b gene) are given. The regions sequenced ~~(Tables 1 and 2)~~ of both clones are ~~indicated~~ provided in Figures 2A-2D (Enod2a; DNA is SEQ ID NO:1; encoded polypeptides are SEQ ID NO:2 and SEQ ID NO:3) and Figures 3A-3D (Enod2b; DNA is SEQ ID NO:4; encoded polypeptides are SEQ ID NO:5 and SEQ ID NO:6). The region of approximately 100% homology between the two genomic clones ~~is indicated~~, as ~~are~~ well as the regions of the clones homologous to the Enod2 cDNA clone, can be determined by alignment. Restriction endonucleases are labelled as follows: H=HindIII, B=BamHI, S=Sau3A, E=EcoRI.

Please add the following paragraphs following Paragraph [0024] at page 14, line 17:

[0024a] FIG. 2A-D provides the nucleotide sequence of the Enod2a genomic clone (SEQ ID NO:1) and the amino acid sequences for SEQ ID NOs:2-3.

[0024b] FIG. 3A-D provides the nucleotide sequence of the Enod2b genomic clone (SEQ ID NO:4) and the amino acid sequences for SEQ ID NOs:5-6.

[0024c] BRIEF DESCRIPTION OF THE SEQUENCES

[0024d] SEQ ID NO:1 is the nucleotide sequence of the Enod2a genomic clone in FIG. 2A-D.

[0024e] SEQ ID NO:2 is the amino acid sequence encoded by nucleotides 1654-2494 of SEQ ID NO:1.

[0024f] SEQ ID NO:3 is the amino acid sequence encoded by nucleotides 1751-2678 of SEQ ID NO:1.

[0024g] SEQ ID NO:4 is the nucleotide sequence of the Enod2b genomic clone in FIG. 3A-D.

[0024h] SEQ ID NO:5 is the amino acid sequence encoded by nucleotides 2456-3264 of SEQ ID NO:4.

[0024i] SEQ ID NO:6 is the amino acid sequence encoded by nucleotides 2553-3380 of SEQ ID NO:4.

Please amend Paragraphs [0026]-[0027] on page 14, line 22, through page 17, line 7, as follows:

[0026] The Enod2 gene described herein is an early nodulin gene of soybean (Glycine max), which encodes nodulin polypeptides with an apparent molecular weight of about 75 kDa, nodulin 75 (N-75). Two such genes are exemplified by the Enod2a and Enod2b genes which are identified by the DNA sequences given in ~~Tables 1 and 2~~ Figures 2A-D and 3A-D, respectively.

[0027] The Enod2 regulatory region is the DNA sequence 5' and adjacent to the Enod2 coding sequence, which includes promoter sequences and promoter-associated sequences and controls tissue-specific expression of the Enod2 genes in soybean. The regulatory region extends about 1 kb upstream from the transcription start site of an Enod2 gene. All the signals required from tissue-specific regulated gene expression are contained in the approximately 1 kb 5' flanking region. Within this stretch of DNA are sequences with homology to the TATA and CAAT consensus sequences of eukaryotic promoters, and the nodulin gene consensus sequences a and c (V. P. Mauro et al. (1985), supra), which are believed to be involved in the regulation of the expression of nod genes expressed later than Enod2 during nodulation. There are also sequence motifs with homology to the SV40 enhancer core consensus sequence which are found in the regulatory region of the soybean Enod2a gene. There may also be other sequence elements which modulate the level of gene expression, which respond to stimuli from the B. japonicum, or which determine the tissue-specific expression in the developing soybean root nodule after inoculation with Bradyrhizobium japonicum. The expression of Enod2 genes controlled by the Enod2 regulatory region is tissue-specific in that it is limited to the cortex of developing soybean root nodules. The Enod2 regulatory region controls early gene expression in the developing root nodule of soybean with expression beginning at about 7 days after seed planting and inoculation. Expression is induced by contact with soybean nodulating bacteria, such as B. japonicum. Enod2 gene expression also occurs in the ineffective nodules induced by strains of Rhizobium fredii. The Enod2a regulatory region is a DNA sequence which includes promoter sequences and promoter-associated sequences and controls the expression of the soybean Enod2a gene. The Enod2a regulatory region extends about 1 k:b upstream from the Enod2a gene transcription start. This region is specifically identified by the DNA sequence in ~~Table 1~~ Figure 2A-D from about

nucleotide 520 to about nucleotide 1565. The Enod2b regulatory region is a DNA sequence which includes promoter sequences and promoter-associated sequences and controls the expression of the soybean Enod2b gene. The Enod2b regulatory region extends about 1 kb upstream from the Enod2b gene transcription start. This region is specifically identified by the DNA sequence in ~~Table 2~~ Figure 3A-D from about nucleotide 1320 to about nucleotide 2365. These regulatory regions direct tissue-specific expression of a downstream structural gene, such that the gene is selectively expressed in the inner cortex of the developing root nodule in soybean. The Enod2 common regulatory region is the DNA sequence extending about 500 bases upstream of the transcription start site of an Enod2 gene. The Enod2 common regulatory region is exemplified by the homologous sequences of Enod2a and Enod2b extending from about nucleotide 1050 to about nucleotide 1565 (~~Table 1~~ Figure 2A-D), and about nucleotide 1850 to about nucleotide 2365 (~~Table 2~~ Figure 3A-D), respectively. This common regulatory region controls tissue-specific expression of downstream genes in the cortex of developing soybean root nodules.

Please amend Paragraph [0035] at page 22, line 5, through page 23, line 6, as follows:

pEnod2 was isolated from a cDNA library prepared with 21-day-old soybean root nodule RNA, using RNA from 10-day-old nodules as a probe. Thus, pEnod2 represents an early nodulin cDNA clone. The early nodulin encoded by pEnod2 was identified by hybrid-selecting nodule mRNA and translating in vitro. Two polypeptides, with apparent Mrs of 75000, were found and were each called N-75. The mRNAs homologous to pEnod2 were only about 1200 nucleotides long, with the capacity to encode a protein of at most about 45 kDa. Therefore the soybean-specific insert of pEnod2 was sequenced and the amino acid sequence of N-75 was deduced. Two ORFs of similar size were found (labelled ORF1 and ORF2 on ~~Tables 1 and 2~~ Figures 2A-D and 3A-D), one with about 20 methionines and the other a proline-rich sequence, with a repeating heptameric sequence. Because of the anomalous migration on SDS-polyacrylamide gels and because of the labelling patterns the two N-75s, it was concluded that the proline-rich coding sequence (ORF1) was that of N-75. It is believed that N-75 is involved in nodule morphogenesis because of its proline content and because of the pattern of expression in the

developing nodule. N-75 appears at about day 7 after sowing and inoculation, and increases through day 13; mRNA continues to be present at least through day 21. N-75 is also produced in the developing ineffective nodule of soybean inoculated with Rhizobium fredii USDA257. That leads to the conclusion that typical nodule structure with successful infection of the root by rhizobia is not absolutely required for Enod2 expression.

Please amend Paragraph [0037] at page 23, line 14, through page 24, line 3, as follows:

Two soybean genomic clones corresponding to pEnod2 have been isolated and the DNA sequences of the coding and flanking regions have been determined (~~Tables 1 and 2~~ Figures 2A-D and 3A-D). The genes, termed Enod2a and Enod2b, are essentially homologous from about 600 bp 5' to the ATG translation start codon through the coding region, which is not interrupted by introns, and through some 500 bp of 3' flanking sequence. Comparison of the genomic clones with the Enod2 cDNA sequence indicates that one or both of these genes are expressed in the developing root nodule. S1 mapping of the transcription start site led to the conclusion that the Enod2a start site is at nucleotide 1543 .+-.20 as shown in ~~Table 1~~ Figure 2A-D, and the Enod2b start site is deduced to be similarly located at about nucleotide 2350, as shown in ~~Table 2~~ Figure 3A-D.

Please amend Paragraphs [0054]-[0056] at page 36, line 4, through page 37, line 18, as follows:

[0054] Subsequently, portions of p4.5BE and p10.2 were subcloned into pUC18 and pUC19 vectors and sequenced as described in Example 2. The DNA sequences of the portions of p4.5BE and p10.2 containing the Enod2 genes are displayed in ~~Tables 1 and 2~~ Figures 2A-D and 3A-D. The coding regions and the deduced amino acid sequences of both genes are shown.

EXAMPLE 4

Sequence Analysis of the Enod2a and Enod2b Genes of Soybean

[0053] Standard techniques, as described above, were used for the sequencing of the Enod2a and Enod2b genomic sequences. The coding region of each of these genes is an uninterrupted sequence of 930 bp. ~~Table 1~~ Figure 2A-D gives the DNA sequence of the coding region of the

Enod2a gene along with about 1650 bp of 5' flanking sequence and about 360 bp of 3' flanking sequence. The coding region and about 600 of 5' flanking sequence of the Enod2b gene is almost identical in sequence to that of the Enod2a gene as shown in ~~Table 2~~ Figure 3A-D; a total of about 2450 of 5' flanking sequence and about 470 bp of 3' flanking sequence of the Enod2b gene are also presented in ~~Table 2~~ Figure 3A-D. It was noted that the two genes were 100% homologous over the coding regions, and almost 100% homologous in the approximately 600 bp of 5' flanking DNA extending to a Sau3A site at positions 1048 in Enod2a and 1852 in Enod2b, and in the 3' flanking DNA that has been sequenced.

[0056] Analysis of the sequence of the cDNA clone pEnod2 and the sequences revealed that there were two open reading frames (ORF1 and ORF2) of similar length; both are noted in ~~Tables 1 and 2~~ Figures 2A-D and 3A-D. The anomalous migration in SDS-polyacrylamide gel electrophoresis experiments led, in part, to the conclusion that the ORF1 is the actual coding sequence of the Enod2 genes encoding N-75. The polypeptide encoded by ORF1 is rich in proline, and proline-rich polypeptides are known to exhibit aberrant behavior during SDS-polyacrylamide gel electrophoresis (J. W. Freytag et al. (1979), supra). The second line of reasoning was that one of the hybrid-selected translation products was devoid of methionine; ORF1 has only one methionine codon (at the translation start) while the alternate ORF1 contained about 20 methionine codons, and therefore its translation product should have been labelled readily with ^{35S}-methionine.

Please replace Figures 2A and 3A with replacement Figures 2A and 3A, which are attached following the Remarks section along with annotated Figures 2A and 3A showing changes made.

JOINT DECLARATION FOR PATENT APPLICATION

As the below named inventors, we hereby declare that:

Our residences, post office addresses and citizenship are as stated below next to our names;

We believe that we are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled

ENOD2 GENE REGULATORY REGION , the specification of which

___ is attached hereto.

X was filed on July 1, 1988 as Application Serial No. 214,297 and was amended on _____ (if applicable).

We hereby state that we have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

We acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

We hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application(s) for patent or inventor's certificate having a filing date before that of the application on which priority is claimed;

Prior Foreign Application(s)

Country	Application No.	Date of Filing (day,month,year)	Date of Issue (day,month,year)	Priority Claimed Under 35 U.S.C. 119
				Yes ___ No ___
				Yes ___ No ___
		NONE		Yes ___ No ___
				Yes ___ No ___

We hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims provided by the first paragraph of Title 35, United States Code, §112, we acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application;

Application Serial Number	Date of Filing (day,month,year)	Status - Patented, Pending, Abandoned
	NONE	

And we hereby appoint, both jointly and severally, as our attorneys with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith the following attorneys, their registration numbers being listed after their names: Lorrance L. Greenlee, Reg. No. 27,894; Sally A. Sullivan, Reg. No. 32,064; and Ellen P. Winner, Reg. No. 28,547.

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopradize the validity of the application or any patent issuing thereon.

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